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BIOCHEMICAL AND SENSORY ASSESSMENT OF ORDINARY VIRGIN OLIVE OIL

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Abstract

The aim of this study was to make sure that there are no health issues for ordinary virgin olive oil (OVOO) category, through a biological study. Twenty-four male Albino rats weighting approximately 200 grams were divided into four groups, each group containing six rats. Group G1 fed on basal diet as a control negative group. The fat was replaced in the other three groups as follow: group G2 fed on basal diet with extra virgin olive oil (EVOO), group G3 fed on basal diet with OVOO and group G4 fed on basal diet with olive oil (OO) (mixture of virgin olive oil and refined olive oil). The experimental period was 60 successive days. At the end of the experiment lipid profile, liver functions, kidney functions and the activities of SOD, CAT, GSPx, GSH and MAD levels were determined. Undisputedly, EVOO had the best results in all study. OVOO did not affect negatively on all parameters unlike olive oil (contains refined olive oil) which caused significant increases in TC, LDL, VLDL, TG and MAD levels and also increased ALT, AST, ALP, SOD, CAT GSPx activity whereas HDL and GSH levels were decreased. Moreover, OVOO did not show any histopathological alterations in examined liver sections.

Keyword :EVOO, OVOO, refined olive oil, chemical and physical properties, fatty acid composition, phenolic component, glucose, lipid profile, liver function, kidney function, oxidative stress, histopathology**.**

Introduction

Olive oil is one of the oldest vegetable oils, mainly produced in Mediterranean countries. Its consumption is increasing throughout the world due to the growing interest in Mediterranean diet, of which olive oil is one of the main ingredients and which is often strongly correlated with the reduction of cardiovascular diseases and certain forms of cancer **(Khlil *et al.*, 2017).** The International Olive Council **(IOC) standards (2019)** and the **Codex Alimentarius (2003)** distinguish four categories of virgin olive oil: EVOO, virgin olive oil (VOO), OVOO and lampante virgin olive oil (LVOO). The OVOO category is part of the four virgin olive oil categories in the **IOC Trade Standard (2019)** and **Codex standards (CXS 33-1981)**. It is defined as an ordinary virgin olive oil, having a degree of acidity ≤ 3.3% and/or a median of defects ≥ 3.5 according to the IOC standard and 2.5 according to the Codex.

Since October 2017, in codex committee **CCFO25th (2017),** an electronic working group (EWG) was created within the Codex and chaired by Spain. EWG has widely discussed the eventual removal of OVOO category of from the Codex standard and no consensus has been reached.

In **CCFO26th (2019)**, most of the countries supporting the removal of this category of oil have their argument for the protection of consumer safety considering the low quality of this oil

category, and the allowed limits of acidity up to 3.3% and median of defect up to 6. In terms of consumer protection, it does not represent a food safety issue and there is no scientific evidence to show that this category of oils compromises consumer health. The acidity parameter is not directly linked to toxicological problems but, it is sometimes an indicator of lack of quality (alteration by hydrolysis of fatty substances). According to **CODEX STAN 210-1999**, this quality factor is supplementary information to the essential composition and quality factors.

Most of the scientific papers on olive oil focus on health implications of EVOO. However, as yet, there are still no well-founded studies proving the toxicity or harmfulness of OVOO. Although, considerable number of consumers use it without any problem or feeling bad effect on health in many regions in Morocco, Algeria and Egypt, for example.

The debate on the eventual removal of the OVOO category from the Codex standard is ongoing within the Codex electronic working group and a consensual conclusion is very awaited. But, during the development or revision of a standard, it is crucial to consider economic and normative aspects related to the product. There are many aspects that should be considered during the development or revision of a standard:

According to categories of olive oil production, EVOO remains the lead product, although other categories such as VOO and OVOO are also of heavy consumption, especially in many producer countries. According to the IOC statistics for the last years, the production of olive oil is about 3 million tonnes covering 4 categories, namely EVOO, VOO, OVOO, and LVOO. This classification lacks quantitative precision due to the absence of denomination of the OVOO category in some producer countries. However, in OVOO producer and consuming countries, the share of production of this latter stands at almost 20% to 30% depending on the country and the production year. In Iran for example, an average of 3000 tonnes of OVOO is produced every year, representing about 30% of its production. In Egypt, Algeria, and Morocco, OVOO represents 10% to 20% of their productions.

With regard to international trade of OVOO, the ordinary virgin olive oil is exported as such, because there is a need and a market for this quality. Since 80 thousand tonnes of OVOO are marketed every year, not only in traditionally producer countries but also is some Middle-East and African countries. Also, one of the strengths of this category of oil is its current presence in several national and international standards, notably in those of the IOC and the Codex. The removal without transition, of this category of oil from the Codex standard will create some difficulties for producers as well as for its commercialization. Also, the removal of this category of oil from the Codex standard will increase the divergence between different international standards, notably those of the IOC and the Codex, which is against the Codex directives and objectives to enhance coordination among all works on food standards undertaken by international governmental and non-governmental organizations.

Although the Codex is trying to remove this category of olive oil (OVOO), the refined olive oil category is still produced from low quality olive oil and undergoes a refining process that removes most of the free fatty acids, considered an undesirable component of oils, and other impurities in the oil. Potentially beneficial substances, such as polyphenols, are also removed during the refining process. The resulting oil is tasteless, colorless, and odorless, similar to commonly consumed refined vegetable oils **(ISO 2016)** still exists.

So, our target was to make sure that there are no health issues for OVOO category through a biological evaluation for lipid profile levels, oxidative stress, liver function, kidney function and histopathological examination of liver.

Materials and Methods

Oil samples: EVOO and LVOO were obtained from Research and Training Olive Oil mill, Food Technology Research Institute (FTRI), ARC. Olive oil category is a mixture from extra virgin olive oil and refined olive oil.

Kits: Kits of glucose; lipid profile (TC, LDL, HDL and TG); liver function (ALT, AST and ALP); kidney function (creatinine, urea and uric acid), SOD, Catalase, GSPx, GSH and MDA were obtained from Biodiagnostic Co., Doki, Giza, Egypt.

Quality characteristics: Peroxide value (PV) and free fatty acids% (FFAs) were evaluated following the methodology proposed by **A.O.A.C. (2019).** UV spectrophotometric indices (K232, K270, and ΔK) were measured according to **the EEC 2568/ (1991) Regulation methods.**

Fatty acids composition analysis and determination were carried out by preparation of methyl ester according to **(Cossignani *et al.*, 2005)** followed by the identification of methyl esters using an Agilent 6890 series gas chromatograph apparatus equipped with a DB23 column (60 m X 0.32 mm X 0.25 μm)) **(Eid and Elsorady, 2012)**.

Total phenols content was determined according to the method described by **Gutfinger (1981),** and results were expressed as mg of caffeic acid per kg of oil.

Phenolic compounds of oil samples were identified and determined using HPLC following the method described by **Goupy *et al.* (1999).**

Total pigments (Chlorophyll and Carotenoids) were determined as follow: 7.5 g of olive oil was accurately weighed and dissolved in cyclohexane up to a final volume of 25 ml. Then, Chlorophyll and Carotenoids were determined by their absorption at 670 and 472 nm, respectively, using a spectrophotometer (JENWAY 6405 UV/Vis., England) **(Minguez-Mosquera *et. al.*, 1991).** Results were expressed (mg/kg oil) as pheophytin and β-carotene for chlorophyll and carotenoids, respectively.

The unsaponifiable matter percentage was determined as method stated in **AOAC (2019).**

Organoleptic Evaluation: The organoleptic evaluation was assessed for the EVOO and OVOO according to the International Olive Oil Council **(IOOC, 2019).**

Refining process

Neutralization of the oil, decolorization of neutralized oil were done by the method of **(EEC 2568/1991 Regulation methods)**. Deodorization procedure was occurred according to **Pardun, (1988).**

Animals: Twenty-four male Albino rats, average weight of 200 g $\pm$ 5 g. were obtained from the animal house of the Food Technology Research Institute, Agriculture Research Center, Giza, Egypt. The rats were housed in wire cages at normal healthy laboratory condition (temperature was adjusted at 25 ± 2 °C for 12-hour light – dark animals were adapted on free access of water) and were fed for one week on basal diet before the initiation standard of the experiment.

The composition of the basal diet (g/100g): Casein 10%; cellulose 5%; fresh vegetable oil (corn oil) 10%; corn starch 70%; salt mixture 4% and vitamin mixture 1%, according to; **Aletor, 1993** and **Aning *et al.*, 1998.**

Experimental design: Animals were divided into four equal groups six rats each. The first group of rats (G1) was fed on basal diet and was considered as negative control group (normal control). Corn oil was replaced in the other three groups. Rats of the second group (G2) fed on basal diet mixed with EVOO at the same oil ratio in basal diet. Group (G3) fed on basal diet mixed with OVOO at the same oil ratio in basal diet. Group (G4) fed on basal diet mixed with olive oil (mixture of EVOO and refined olive oil) at the same oil ratio in basal diet. The experimental period was 60 successive days. Food consumption was monitored, and weekly was weight and determined.

Growth of rats: The gained weight was calculated by the final body weight – the initial body weight**.**

Feed efficiency ratios (FER) = body weight gain (g) / food intake (g) according to **Chapman *et al.*, (1959).**

Biochemical assay: After 60 days (the period of experiment), the blood samples were collected from the eye plexuses of the animals. Then they were divided into two parts. The first one was put in a dry clean centrifuge glass tube without any coagulation to prepare serum. The samples were left for 15 minutes at normal temperature room, after that the tubes were centrifuged for another 15 minutes at 300 rpm. From then until the time of analysis the clean supernatant serum was kept frozen at -20 °C.

Serum glucose was measured according to **Kaplan, (1984).**

Lipid profile:
Serum total cholesterol was determined according to the colorimetric method described by **Allain *et al.*, (1974).**
Serum high density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c) were determined according to **Assmann, (1979)** and **Lee and Nieman, (1996),** respectively**.**
Serum triglycerides (T.G) were measured using the modified kinetic method according to the method described by **Fossati and Prencipe, (1982).**
Serum very low-density lipoprotein cholesterol (VLDL-c) was calculated as mg/dl according to **Wallach, (1992)** using the equation: VLDL-C concentration mg/dl = TG/5

Liver function: Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were described method of **Bergmeyer and Harder, (1986)**. Alkaline phosphatase activity (ALP) was measured by absorbance at 405 nm of paranitrophenol which was formed from para-nitrophenylphosphate as a substrate using the method of **Varley *et al.*, (1980).**

Kidney function:
Creatinine, urea and uric acid were measured using the method of **Henry (1974)**, **Fawcett and Scott (1960)** and **Caraway, (1955),** respectively.

Blood enzymes activity:
The second part of blood samples (erythrocyte) was used to measure superoxide dismutase (SOD), glutathione reduced (GSH), glutathione peroxides (GSPx) and catalase (CAT)

calorimetrically according to the method of **Nishikimi *et al.*, (1972), Ellman, (1959)**, **Rotruck *et al.*, (1973)** and **Aebi, (1984),** respectively.

The activity of lipid peroxidation level Malondialdehyde (MAD) was determined in serum by the colorimetric method described by **(Meltzer *et al.*, 1997).**

Histopathological examination: At the end of the experiment liver samples of the rats of all groups were carefully removed, washed in saline solution, dried between 2 filter papers and immediately weighted, collected and fixed in 10% neutral buffered formalin, the samples were dehydrated in alcohol, cleared in xylol and embedded in paraffin. 4µ thick Hematoxylene and eosin-stained sections were prepared (**Yoon *et al.*, 2001**). Histopathological examination was done in histopathology department, Faculty of Veterinary Medicine, Cairo University.

Relative organ weight was determined according to **Chapman *et al.*, 1959** using the following equation:
Relative liver weight % = [liver weight (g)/final body weight (g)] x 100

Statistical analysis: The obtained results were subjected to statistical analysis using the standard analysis of variance as outlined by **Snedecor and Cochran, (1980)**

Results and Discussion

1-Chemical properties of different olive oil categories:

Results in Table (1) show that, the lowest significant values of free fatty acids (FFA%) were found in refined olive oil category (0.11%) and olive oil category (0.16%). On the other hand, OVOO had the highest significant value of FFA% (2.88%). FFA% value was 0.49%EVOO.

Concerning peroxide value (PV) meq O_2/kg oil, it was ranged from (3.3-7.3 meq O_2/kg oil). The highest significant value was found in OO category followed by OVOO (5.21%). On the other hand, the lowest value was recorded in EVOO followed by value of refined olive oil (3.41 meq O_2/kg oil). The values of FFA% and PV for these categories were in the limits that were reported by **IOC (2019).**

The absorbency at 232 nm is caused by hydroperoxides (primary stage of oxidation) and conjugated dienes (intermediate stage of oxidation) while, the absorbency at 270 nm is caused by carbonylic compounds (secondary stage of oxidation) and conjugated trienes (technological treatments) and ΔK index were illustrated in Table (1). EVOO had the lowest significant values of K_{270} (0.105) and ΔK (0.0085) followed by OVOO 0.19 and 0.0099 at the same wavelengths, respectively. The highest significant values of K_{270} and ΔK were found in refined olive oil 1.07 and 0.13, respectively followed by olive oil. There were no significant differences in values of K_{232} in all olive categories. K_{232}, K_{270} and ΔK were in the range stated by **IOC, (2019).**

With respect to oxidative stability (in hours), Data in Table (1) show that EVOO had the highest oxidative stability (28.4h) followed by OVOO (22h) followed by OO (18.5h) on the other hand, refined olive oil had the lowest oxidative stability (13h). The differences of oxidative stability were significant in all olive oil categories. **Basuny *et al.*, (2008)** had found correlation between stability and the bioactive components and the quality indices and their results indicated that the compound most related to oxidative stability were the phenolic compounds and pigments. **Reboredo-Rodríguez *et al.*, (2015)** reported that oxidative stability of olive oil is mainly due to its high levels of MUFA (especially oleic acid content). So, refined olive oil had the lowest oxidative stability due to remove most bioactive components during refining process.

Table (1): Chemical properties (FFA%, PV meq O2/kg, UV absorbency, oxidative stability), UNSAP%, Chlorophyll content (ppm) and Carotenoids content (ppm) of different olive oil categories

Category / Parameter	Extra virgin olive oil	Refined olive oil	Ordinary olive oil	Olive oil	LSD
FFA%	0.49^{b}±0.02	0.11^{c}±0.01	2.88^{a}±0.08	0.16^{c}±0.01	0.0787
PV	3.30^{c}±0.1	3.41^{c}±0.2	5.21^{b}±0.09	7.30^{a}±0.17	0.2794
K232	2.286^{a}±0.011	2.357^{a}±0.2	2.301^{a}±0.2	2.504^{a}±0.101	0.2829
K270	0.105^{d}±0.004	1.07^{a}±0.07	0.19^{c}±0.02	0.451^{b}±0.01	0.0692
ΔK	0.0085^{b}±0.0011	0.13^{a}±0.02	0.0099^{b}±0.0004	0.11^{a}±0.01	0.0210
Induction period (hr)	28.4^{a}±0.7	13.0^{d}±0.3	22.0^{b}±00.3	18.5^{c}±0.4	0.8576
UNSAP%	1.1^{a}±0.05	0.75^{c}±0.05	1.04^{a}±0.04	0.93^{b}±0.03	0.0815
Chlorophyll	3.68^{a}±0.18	0.95^{d}±0.02	2.80^{b}±0.2	1.77^{c}±0.02	0.2547
Carotenoids	2.0^{a}±0.11	0.78^{c}±0.03	1.83^{a}±0.10	1.33^{b}±0.05	0.2064

-Means, within the same row, followed by the same letter are not significantly different
-UNSAP: Unsaponifiable matter

The unsaponifiable fraction (UNSAP%) that represents about 2% of olive oil is composed of a very large number of minor compounds, very important for the flavour and the nutritional properties of EVOOs **(Boskou *et al.*, 2006)**. Data in Table (1) show that UNSAP% ranged from 0.75 – 1.10%. The highest significant percentages were found in EVOO followed by OVOO. On the other hand, refined olive oil had the lowest value.

The color of olive oil varies from a light gold to a rich green oil is due to the presence of natural pigments belonging to the class of carotenoids, chlorophylls, and their derivatives. These substances, other than being responsible for the color, an important qualitative feature of the oil, have antioxidant and, more generally, nutraceutical properties and their quantification can be related to the product's quality and authenticity **(Borello and Domenici, 2019)**. Our results (Table 1) revealed that chlorophyll and carotenoids concentration of olive oil categories under investigation ranged from (0.95-3.68ppm) and (0.78-2.0ppm) respectively. EVOO had the greatest significant values of them on the other hand; refined olive oil had the lowest values of them. The concentration of chlorophyll and carotenoids were 2.8ppm and 1.83ppm respectively in OVOO followed by OO.

Table (2): Sensory attributes of EVOO and OVOO

Category / Attribute	Extra virgin olive oil	Ordinary olive oil
Fruity	4.50	ND
Bitter	2.00	ND
Pungent	1.50	1.00
Defects	ND	Fusty 5.00

-ND: not detected

The sensory attributes for EVOO and OVOO are shown in Table (2). The median (Me) of the positive (fruity attribute) recorded a high value in EVOO according to sensory characteristic limits for EVOO of **IOC, 2019** (Fruity ≥ 0.00 and defects=0.00). Moreover, the Me of fusty

negative attribute recorded in OVOO with value (5.0) which was in the limits of IOC Standard, 2019 (Median of defects; 3.5 ≥ Me < 6 and fruity = 0.00). Sensory analysis of oil, meaning the official organoleptic assessment of olive oil respectively the panel test (PT), relies on the standards of the International Olive Council (IOC), furthermore as on the Regulation of the European Commission (EC). The classification of olive oil categories is relied on these regulations as EVOO, VOO or LVOO, that but isn't comfortable to obviously discriminate between totally different quality levels inside the grade EVOO. Also, it is used to determine if oils contain one or more of the defects that commonly occur in oils from improper fruit storage, handling, pest infestation, oil storage, or processing problems **(IOC, 2018)**.

Table (3) Relative percentage of fatty acids of different olive oil categories

Relative % of FA	Extra virgin olive oil	Refined	Ordinary olive oil	Olive oil	LSD 0.05 =
$C_{16:0}$	$15.51^{a}\pm0.06$	$15.54^{a}\pm0.01$	$14.25^{c}\pm0.02$	$15.33^{b}\pm0.03$	0.0665
$C_{16:1}$	$1.24^{c}\pm0.01$	$1.44^{a}\pm0.04$	$1.12^{d}\pm0.01$	$1.34^{b}\pm0.04$	0.05489
$C_{17:0}$	$0.06^{a}\pm0.0$	$0.07^{a}\pm0.002$	$0.06^{a}\pm0.0$	$0.06^{a}\pm0.001$	0.0096
$C_{17:1}$	$0.10^{a}\pm0.01$	$0.11^{a}\pm0.01$	$0.10^{a}\pm0.02$	$0.11^{a}\pm0.0$	0.0230
$C_{18:0}$	$2.36^{d}\pm0.06$	$2.45^{c}\pm0.005$	$2.96^{a}\pm0.04$	$2.57^{d}\pm0.03$	0.0736
$C_{18:1}$	$69.88^{a}\pm0.12$	$66.08^{b}\pm0.58$	$70.34^{a}\pm0.11$	$67.98^{b}\pm0.12$	0.5782
$C_{18:2T}$	-	$0.16^{a}\pm0.01$	-	$0.07^{b}\pm0.005$	0.0105
$C_{18:2}$	$9.00^{d}\pm0.2$	$12.25^{a}\pm0.13$	$9.29^{c}\pm0.03$	$10.67^{b}\pm0.026$	0.2288
$C_{18:3}$	$0.9^{c}\pm0.01$	$0.99^{a}\pm0.02$	$0.85^{d}\pm0.02$	$0.95^{b}\pm0.02$	0.0339
$C_{20:0}$	$0.47^{b}\pm0.02$	$0.47^{b}\pm0.01$	$0.54^{a}\pm0.01$	$0.46^{b}\pm0.03$	0.0364
$C_{20:1}$	$0.35^{a}\pm0.0$	$0.31^{b}\pm0.01$	$0.33^{ab}\pm0.005$	$0.33^{ab}\pm0.02$	0.0253
$C_{22:0}$	$0.13^{b}\pm0.001$	$0.13^{b}\pm0.005$	$0.16^{a}\pm0.002$	$0.13^{b}\pm0.01$	0.0108
TSFA	$18.53^{b}\pm0.03$	$18.66^{a}\pm0.03$	$17.97^{c}\pm0.03$	$18.55^{b}\pm0.05$	0.0678
TUSFA	$81.47^{b}\pm0.12$	$81.34^{b}\pm0.13$	$82.03^{a}\pm0.3$	$81.45^{b}\pm0.05$	0.3294
MUSFA	$71.57^{a}\pm0.28$	$67.94^{c}\pm0.22$	$71.89^{a}\pm0.11$	$69.76^{b}\pm0.13$	0.3715
PUSFA	$9.90^{d}\pm0.1$	$13.40^{a}\pm0.1$	$10.14^{c}\pm0.04$	$11.69^{b}\pm0.06$	0.1494
USFA/SFA	$4.40^{b}\pm0.05$	$4.35^{b}\pm0.09$	$4.56^{a}\pm0.02$	$4.39^{b}\pm0.04$	0.1031
MUSFA/PUSFA	$7.23^{a}\pm0.03$	$5.07^{d}\pm0.06$	$7.09^{b}\pm0.09$	$5.96^{c}\pm0.06$	0.1093

-Means, within the same row followed by the same letter are not significantly different
-TSFA: Total saturated fatty acids
-TUSFA: Total unsaturated fatty acids
-MUSFA: Monounsaturated fatty acids
-PUSFA: Polyunsaturated fatty acids

Concerning the fatty acid composition, the results in Table (3) illustrated that, oleic acid (18:1) as a monounsaturated fatty acid (MUSFA) was the major fatty acid in all olive categories. OVOO and EVOO had the highest significant percentage of oleic acid (70.34%) and (69.88%) respectively, followed by olive oil (67.98%). On the other hand, refined olive oil had the lowest percentage of oleic acid (66.08%). The high content of oleic acid in olive oil serves to slow down penetration of fatty acids into arterial walls **(Mailer, 2006)**. Oil with higher (MUFAs) and lower saturated fatty acids (SFAs) are preferred because of the proven beneficial effect of MUFAs on serum cholesterol levels **(Hashempour *et al.*, 2010).** Palmetic acid (16:0) which represents the major unsaturated fatty acid (USFA) ranged between 14.25-15.51, its greatest significant contents were found in refined olive oil followed by EVOO on whereas, its

lowest percentage was detected in OVOO. In addition, linolenic acid (18:3) contents were found less than 1% in all olive oil categories.
The most important change in the fatty acids' composition was finding of C18:2T in refined olive oil (0.16%) and OO (0.07%). Trans-fatty acids arise during refining of vegetable oils as well as during hydrogenation, or from attempts to eliminate the sterol fraction of seed oils with a fatty acid composition similar to that of olive oil **(Firestone, 2005).**
The results of fatty acids profile were an emphasis that the high result values of physicochemical properties for absorbency in UV at K_{270} for refined olive oil and virgin olive oil in contrary with EVOO and OVOO.

The MUSFA/PUSFA ratio is frequently used as a stability parameter and, in previous studies; the cultivar with higher ratios were those with higher oxidative stability **(Beltran, 2000)**. EVOO had the highest significant value of it (7.23%) in accordance with their higher oxidative stability (30.5 h) followed by OVOO (7.09%) whereas, refined olive oil had the lowest ratio 5.07 with considerable low oxidative stability 13.0h.

Table (4): Total phenol content (mg/Kg as Caffeic acid) and Phenolic compounds of different categories of olive oil

Phenols (mg/Kg)	Extra virgin	Refined	Ordinary	Olive oil	LSD 0.05
Pyrogallol	**174.01^{a}±1.3**	**15.04^{d}±0.14**	**139.52^{b}±1**	**79.93^{c}±2.01**	**2.3214**
Gallic	**33.66^{a}±2.42**	**2.89^{d}±0.43**	**18.28^{b}±1.28**	**8.48^{c}±0.48**	**2.6479**
3-OH Tyrosol	**307.21^{a}±6.1**	**22.14^{d}±0.86**	**166.68^{b}±3.68**	**86.92^{c}±2.02**	**7.0207**
Catechol	**222.32^{a}±6.1**	**14.61^{d}±1.11**	**173.47^{b}±4.07**	**65.51^{c}±3.00**	**7.5324**
4-aminobenzoic	**0.87^{a}±0.02**	**0.01^{d}±0.001**	**0.34^{b}±0.01**	**0.12^{c}±0.02**	**0.0282**
Catechein	**0.96^{a}±0.02**	**0.12^{d}±0.01**	**0.59^{b}±0.03**	**0.22^{c}±0.02**	**0.0282**
Chlorogenic	**23.11^{b}±1.00**	**12.67^{d}±1.5**	**17.89^{c}±1.89**	**37.28^{a}±2.28**	**3.2639**
P-OH- benzoic	**7.92^{a}±0.4**	**0.43^{d}±0.03**	**4.68^{b}±0.12**	**1.93^{c}±0.03**	**0.3951**
Benzoic	**30.56^{b}±1.44**	**5.00^{d}±0.44**	**27.78^{c}±1.08**	**36.65^{a}±1.77**	**2.4098**
Caffeic	**157.86^{a}±2.14**	**11.17^{d}±0.76**	**74.52^{b}±3.00**	**55.77^{c}±0.91**	**3.6435**
Vanillic	**14.71^{a}±0.29**	**0.71^{d}±0.09**	**5.21^{b}±0.22**	**3.34^{c}±0.11**	**0.3678**
Caffeine	**4.27^{a}±0.27**	**0.79^{d}±0.02**	**3.03^{b}±0.26**	**2.13^{c}±0.09**	**0.3660**
Ferulic	**0.79^{a}±0.03**	**0.03^{d}±0.002**	**0.47^{b}±0.03**	**0.09^{c}±0.01**	**0.04107**
Salycillic	**1.24^{a}±0.02**	**0.28^{d}±0.01**	**0.86^{b}±0.03**	**0.73^{c}±0.01**	**0.0352**
Oleuropin	**170.53^{a}±2.57**	**14.03^{d}±0.06**	**142.28^{b}±2.59**	**75.64^{c}±0.64**	**3.4902**
Coumarin	**4.1^{a}±0.16**	**0.59^{d}±0.02**	**2.35^{b}±0.05**	**1.73^{c}±0.036**	**0.17409**
Total phenols content (mg/Kg as Caffeic acid)	**685.68^{a}±4.32**	**63.52^{d}±1.73**	**435.01^{b}±3.45**	**209.19^{c}±2.32**	**5.8747**

-Means, within the same row followed by the same letter are not significantly different

Total phenol content and phenolic compounds of different olive oil categories are tabulated in Table (4). The results revealed that EVOO recorded the highest content of total phenolic compound (685.68ppm) followed by OVOO (435.01ppm) and olive oil (209.19ppm). On the other hand, refined olive oil had the lowest value of total phenolic content (63.52ppm). The differences of total phenol content were significant in all olive categories.
Concerning phenolic compounds, results in Table (4) show that sixteen phenolic components were identified in all olive oil samples. 3-OH tyrosol, catechol, pyrogallol, oleuropin and caffeic acid were the predominant phenolic compounds in different olive oil categories. EVOO had the greatest significant contents of the abovementioned compound (307.21, 222.32, 174.01, 170.53 and 157.86 ppm, respectively) followed by OVOO in the same compounds (166.68.

173.47, 139.52, 142.28, and 74.25ppm, respectively). On the other hand, the concentrations of abovementioned components in olive oil were significantly less than that in previous categories (86.92, 65.51, 79.93, 75.64 and 55.77ppm, respectively). It was noticed that refined olive oil had the lowest significant content of all phenolic compounds.

Although olive oil polyphenols present a minor fraction in the composition of olive oil, they seem to be of great importance when it comes to the health benefits, and interest in their biological and potential therapeutic effects is huge **(Karković Marković *et al.*, 2019)**. Hydroxytyrosol, a phenolic alcohol, has been hypothesized to exert wide range of biological effects, cardioprotective, anticancer applications. neuroprotective, antimicrobial, beneficial endocrine, antioxidant and other effects **(Rodriguez-Morato *et al.*, 2015; Parkinson and Cicerale, 2016; Visioli *et al.*, 2002; Tome-Carneiro *et al.*, 2016; Echeverria *et al.*, 2017; Robles-Almazan *et al.*, 2018 and Wani *et al.*, 2018).** The antioxidant and anti-inflammatory effects of oleuropin are considered to be in the basis of its pharmacological activities such as anticancer, cardioprotective, neuroprotective, gastroprotective, hepatoprotective, antidiabetic, ant obesity, radioprotective and other activities **(Hassen *et al.*, 2015).** Recent study found that HT had antioxidant effects via modulating gut microbiota and enhancing the expressions of antioxidant enzymes in mice **(Wang *et al.*, 2021)**. Also, other investigation found that dietary supplementation of HT tended to increase the semen quality, which may be associated with the alterations in gut microbiota and plasma metabolites **(Han *et al.*, 2022)**.

2-Biological results

Olive oil has been well-known to be efficient with many beneficial and varied actions. The current study was implemented to compare the nutritional impact of olive oil categories (EVOO, OVOO and olive oil) in experimental rats to make sure that there are no health issues for OVOO category. The balance between antioxidant defense capacity and oxidative stress determines the capability of humans and animals to oxidative damage. The aging and pathology of many diseases caused by oxidative stress (**Arrigo *et al.*, 2015).**

Effect of different olive oil categories (EVOO, OVOO and olive oil) on initial body weight, final body weight, net gain, net gain%, food intake, food efficiency ratio (FER), and relative liver weight in rats had been summarized in Table (5). Data showed that the initial body weights did not significantly differ among the groups while at the end of the experiment, there were significant increases in body wight gain of group G2 and group G3 (33.12 and 19.7% respectively), food intake (0.93 and 0.42% respectively) and FER (32.12 and 19.33% respectively) comparing with control negative group G1. Meanwhile, group G4 had high significant decreases in body weight gain, food intake and FER (12.36, 0.93 and 11.5% respectively) comparing with group G1. (**Martensson *et al.*, 1990)** reported that GSH is essential for the function and structural integrity of the gut, thus the deficiency of GSH showed severe degradation of jejunum and colonic mucosa accompanied with weight loss and diarrhea. **(Tripoli *et al.*, 2005 and Servili *et al.*, 2014**) reported that EVOO contains several compounds such as phenolic components and flavonoids responsible for antioxidant effect. While olive oil category (contains refined olive oil) lacks to these compounds. **Lopez-Miranda *et al.*, (2010)** conclude that consumption of extra virgin olive oil might exert beneficial effects in the prevention, development and progression of T2D (Type 2 Diabetes mellitus) compared with refined olive oil.

The dietary impacts of different oil categories on liver weight were recorded in Table (5). With regard to relative liver weight of rats, there were no significant differences were found in this parameter among rats feed on basal diet (G1), EVOO (G2), and OVOO (G3) throughout the feeding period 60 days. Our data agreed with **Nogoy *et al.*, 2020** who reported that were no significant differences in relative organs weights after rats feed on EVOO. On the other hand,

group G4 (fed on olive oil which contains refined olive oil) had significant increases in relative liver weights among all tested groups. **Illesca *et al.*, 2015** and **Longhi *et al.*, 2017** reported that higher ingestion of potentially toxic components by the rat fed trans fatty acids lead to higher hepatosomatic index causes liver damage.

Table (5): Effect of olive oil categories on initial weight, final weight, net gain, food intake, food efficiency ratio and relative liver weight in rats

Treat-ments	Initial (g)	Final (g)	Weight gain	Gain %	Food intake (g)	FER	Liver	Liver%
G1	199.80^{a}±1.800	341.45^{c}±6.450	141.60^{c}±4.650	70.88^{c}±1.685	1179^{c}±9.000	0.1200^{bc}±0.003	7.547^{c}±0.650	2.21^{b}±0.150
G2	200.10^{a}±2.100	388.60^{a}±3.600	188.50^{a}±1.500	94.20^{a}±0.240	1190^{a}±15.000	0.1584^{a}±0.001	8.620^{b}±0.390	2.22^{b}±0.080
G3	200.30^{a}±1.300	369.80^{b}±5.700	169.5^{b}±4.400	84.62 ^{b}±1.690	1184^{b}±10.000	0.14320^{ab}±0.003	8.468^{b}±0.950	2.29^{b}±0.200
G4	200.15^{a}±3.150	324.30^{d}±6.300	124.10^{d}±3.150	62.02^{d}±0.585	1168^{d}±13.000	0.1062^{c} ±0.001	11.87^{a}±0.751	3.66^{a}±0.150
LSD	1.405	2.364	2.591	1.685	4.949	0.03594	0.4215	0.0903

- Means, within the same column, followed by the same letter are not significantly different at < 0.05
- Means are followed by the corresponding standard deviation
- G1: fed on basal diet, G2: fed on EVOO, G3: fed on OVOO and G4: fed on OO (Mixture of virgin olive oil and refined olive oil)

Effect of different olive oil categories on serum glucose and lipid profile levels in rats are shown in Table (6). Data revealed that the highest significant increase in blood glucose level was noticed in rats of group G4 (73.44%) while, the increase in group G3 was nonsignificant (4.07%) compared to normal control. On the other hand, there was slightly nonsignificant decreases in serum glucose levels among rats of group G2 comparing with normal control group. The increase in blood glucose that was noticed in group G4 may indicate a disrupted carbohydrate metabolism due to the enhanced breakdown of liver glycogen, possibly mediated by an increase in the adrenocorticotrophic and glucagon hormones and/or reduced insulin activity, as reported by (**Raja *et al.*, 1992)**. Intake of high-fat foods, including trans fatty acids, may have an impact on the formation of free radicals which can induce oxidative stress, inflammatory responses and destruction of pancreatic ß cells. In addition to their effect on membranes and blood glucose levels, trans fatty acids are also associated with systemic inflammation **(Mozaffarian *et al.*, 2006).** Also, **Mozaffarian *et al.*, (2009)** concluded that trans fatty acids (TFA) may also worsen insulin sensitivity, particularly among individuals predisposed to insulin resistance; possible effects on weight gain and diabetes incidence require further confirmation. EVOO consumption in mice showed higher glucose-stimulated insulin secretion, protection against the liver cell, and β-cell membrane lipid peroxidation than the control and sunflower oil groups **(Oliveras-López *et al.*, 2008).** Also, **Schwingshackl *et al.*, (2017)** reported that EVOO consumption might demonstrate a favorable result in the prevention, development, and progression of type2 diabetes mellites compared with refined olive oil. The beneficial role of EVOO might be attributed to apigenin rutinoside, elenolic acid, luteolin, flavonoid, and hydroxytyrosol found in Chetoui EVOO, which can preserve the integrity of the biological membranes **(Serreli and Deiana**, **2020).**

Also, data in Table (6) showed that Group G4 (fed on olive oil category) significantly increased serum TC; LDL-cholesterol; TG and VLDL-cholesterol (33.96, 97.50, 59.06 and 59.06%, respectively) compared to normal rats. While, HDL-cholesterol in the same group (G4) significantly declined (16.74%) compared to normal control. On contrary, there were non-significant differences in serum total TC, HDL-cholesterol, LDL-cholesterol, TG and VLDL-

Table (6): Effect of olive oil categories on serum glucose levels and lipid profile levels in rats

Treatment	Glucose (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLD-C (mg/dl)	T.G (mg/dl)
G1	84.70^{bc}±1.700	89.80^{bc}±3.800	45.40^{ba}±1.400	30.77^{bc}±1.970	13.63^{bc}±0.430	68.15^{bc}±2.150
G2	81.95^{c}±1.450	87.85^{c}±2.150	47.50^{a}±3.750	27.18^{c}±3.286	13.16^{c}±1.703	65.80^{c}±2.697
G3	88.15^{b}±2.150	91.22^{b}±3.60	42.40^{b}±4.350	34.27^{b}±1.600	14.45^{b}±0.850	72.25^{b}±4.250
G4	146.90^{a}±6.900	120.30^{a}±5.250	37.80^{c}±4.800	60.77^{a}±0.630	21.68^{a}±1.080	108.40^{a}±5.010
LSD	4.6420	2.2790	3.2180	3.9500	0.9827	5.1660

- Means, within the same column, followed by the same letter are not significantly different at <0.05.
- Means are followed by the corresponding standard deviation.
- G1: fed on basal diet, G2: fed on EVOO, G3: fed on OVOO and G4: fed on OO (Mixture of virgin olive oil and refined olive oil).

cholesterol among rats feed on EVOO (G2) and OVOO (G3) compared to control group (G1). EVOO and OVOO had the best results' lipid profile in contrary with olive oil and we could explain that as follow; Polyphenol-rich vegetable oils and monounsaturated fatty acids provide protection against an array of human diseases such as cancer, atherosclerosis, and cardiovascular disease (CVD), including those involving the central nervous system. Olive oil, which is known for its healthful properties, which are often attributed to its high monounsaturated fatty acid content, including oleic acid (18:1 n-9), is a prominent member of the family of polyphenol- and monounsaturated fatty acid-rich oils. However, EVOO, unlike other vegetable oils, contains high amounts of several micronutrient constituents, including polyphenolic compounds such as hydroxytyrosol, tyrosol, and oleuropein **(Sirtori *et al.*, 1992).** In vitro and in vivo human and animal studies have shown that EVOO reduces blood pressure **(Perona *et al.*, 2004)**, improves the lipid profile by increasing HDL-cholesterol and reducing LDL-cholesterol and TG levels **(Blanco-Molina *et al.*, 1998; Covas *et al.*, 2006a; and Carluccio *et al.*, 2007)** reduces oxidative stress, and inhibits human lipoprotein oxidation, making LDL, for instance, less atherogenic **(Berrougui *et al.*, 2006 and Covas *et al.*, 2006b)**. Also, there are many studies have shown that consumption of diets rich in OO, which contains important phenolic compounds, has a remarkable ability in reducing cholesterol level and platelet aggregation and is inversely associated with risk of CHD (coronary heart deseases) **(Esfarjani *et al.*, 2013). Atefi *et al.*, (2018)** reported that replacing canola oil and sunflower oil by EVOO may have some beneficial effects on systolic blood pressure, TG and VLDL-Cholesterol in women with type 2 diabetes. In some studies, it is reported that MUFA intake significantly decreases TG, TC and LDL-C levels, also increases serum HDL-C **(Armin *et al.*, 2010 and Terés *et al.*, 2008).** There are multiple mechanisms through which phenolic compounds may have an impact on the development of atherosclerosis, such as the inhibition of LDL oxidation, endothelial expression of the tissue factor and adhesion molecules **(Malekmohammad *et al.*, 2019).** Consumption of trans fats has been showed to increase the risk of coronary heart disease (CHD) in part by increasing the level of LDL referred as "bad cholesterol" and decreases the level of HDL referred as "good cholesterol" and raising TG in

the bloodstream thus promoting systemic inflammation **(Food, Board Nutrition. Institute, 2005)**

Table (7): Effect of olive oil categories on serum liver functions and kidney functions level in rats

Treatments	Liver functions			Kidney functions		
	ALT (U/L)	AST (U/L)	ALP (U/L)	Creatinine (mg/dl)	Urea (mg/dl)	Uric (mg/dl)
G1	26.55^{b}±1.550	29.40^{b}±1.400	68.25^{b} ±1.750	0.4567^{b}±0.122	25.80^{b}±1.500	1.480^{b}±0.180
G2	23.10^{c}±0.600	27.15^{c}±2.150	65.30^{c}±1.300	0.4500^{b}±0.050	24.95^{b}±2.950	1.430^{b}±0.180
G3	27.34^{b}±1.750	30.65^{b}±1.650	70.40^{b}±3.400	0.4802^{b}±0.050	25.85^{b}±2.850	1.560^{b}±0.360
G4	35.60^{a}±2.600	44.35^{a}±3.350	99.55^{a}±4.550	0.7100^{a}±0.110	31.20^{a}±3.200	2.300^{a}±0.800
LSD	1.8030	1.5570	2.6990	0.08037	1.8730	0.5270

- Means, within the same column, followed by the same letter are not significantly different at <0.05
- Means are followed by the corresponding standard deviation
- G1: fed on basal diet, G2: fed on EVOO, G3: fed on OVOO and G4: fed on OO (Mixture of virgin olive oil and refined olive oil)

Effect of different olive oil categories on serum liver functions had been showed in Table (7). Liver Function Tests (LFTs) are used to check liver impairment, evaluate the development of diseases, and monitor the impact of hepatotoxic drugs and necrosis in the liver of animals. Serum aminotransferases and alkaline phosphatase, bilirubin and albumin also the prothrombin time included in (LFTs) (**Macfarlane *et al.*, 2000**). The concentration of intracellular hepatic enzymes that have leaked into the circulation measured by (ALT) and (AST). Moreover, normal functioning of liver indicated by normal levels of ALT and AST, indicators of liver function. When the enzymes leak from the liver cytosol into the blood stream that led to higher level of ALP in serum (**El-Sharouny *et al.*, 2016**), which is indicative of hepatotoxic of activity in rats. Data in Table (7) revealed that EVOO in group G2 significantly reduced the concentration of ALT, AST and ALP enzymes (12.99%, 7.65% and 4.32%, respectively) comparing with control group G1, while group G3 (fed on ordinary olive oil) had no significant differences in the concentration of the previous enzymes. On the other hand, group G4 (fed on olive oil category) significantly induced the concentration of ALT, AST and ALP enzymes (34.09%, 50.8% and 63.4%, respectively) in contrary with control group. Our results agreed with **Shidfar *et al.*, (2018)** who reported that ALT and AST enzymes significantly decreased in the patients' group that received the hypocaloric diet enriched with olive oil (20% of total energy intake) comparing with control group which received the hypocaloric diet with normal fat. In this regard, the high concentration of monounsaturated fatty acids including oleic acid, flavonoids, phenolic compounds like hydroxytyrosol (HT) which found in EVOO and oleiuropein activate various signaling pathways in the hepatocytes. Activation of these signaling pathways results in the prevention of inflammation, oxidative stress, endoplasmic reticulum stress, mitochondrial dysfunction, and insulin resistance leading to prevention or resolution of liver damage **(Sofi *et al.*, 2013).** Also, **Abril *et al.*, (2019)** found that, extra virgin olive oil contains substances that delay the rate of oxidation by directing the breakdown of

peroxides into stable substances that do not promote further oxidation or by sweeping free radicals away.

Elevation in these enzymes in case of olive oil which contains refined olive oil can be used as markers to estimate the extent of liver damage. Our results agreed with **Dhibi *et al.*, (2011)** who noticed a close positive correlation between TFA levels in dietary fat and AST, ALT and ALP. These results revealed hepatic damage in rats consumed TFA. In this concern, it was reported that oxidative stress mediates many of the effects caused by oxidized fats **(Pizzino *et al.*, 2017)**. Oxidative stress results from an imbalance between oxidant production and antioxidant defenses **(Maritim *et al.*, 2003)**. Oxidative stress induced by free radicals has been linked to the development of several diseases such as cardiovascular, cancer, and neurodegenerative diseases **(Halliwell, 1997)**. When cellular antioxidant mechanisms are over whelmed, a long-term decline in their antioxidant capacity causes the oxidative stress **(Videla *et al.*, 2004)** and **(Browning and Horton, 2004)**. Oxidative stress is now believed to be an important factor in the development of non-alcoholic fatty liver disease (NAFLD) **(Videla *et al.*, 2004** and **2006)**. NAFLD is the most common liver disorder in the world, and in obesity, type 2 diabetes and related metabolic diseases, its incidence reaches 70-90%. The disease is characterized by the accumulation of triacylglycerols inside liver cells, and the condition can progress into more serious liver disease, such as non-alcoholic steatohepatitis, liver fibrosis, cirrhosis, and more rarely, liver carcinoma **(Dhibi *et al.*, 2011)**. Also, the formation of reactive oxygen species (ROS) may increase the dietary oxidized fats and may cause an increased damage of proteins in the liver by enhancing lipid peroxidation of the cell membrane and increasing the generation of (ROS) which can lead to calcium homeostasis disturbances, increase membrane fluidity and cell death **(Pizzino *et al.*, 2017)**.

Effect of different olive oil categories on kidney functions were clarified in Table (7) by determination of creatinine, urea and uric acid. Creatine metabolism by muscles produce creatinine as a major waste product of the process. It's filtered in kidneys by the glomerulus and excreted by the tubules. Besides free-creatinine appears in the blood serum (**Foley *et al.*, 2005, Myers *et al.*, 2006** and **Stevenes *et al.*, 2006**), urea and uric acid are the principal waste products of protein catabolism. They synthesized in the liver from ammonia produced as a result of the deamination of amino acids. The rate of production is accelerated by a high protein diet or by increased endogenous catabolism due to starvation or tissue damage (**Ozer *et al.*, 2014**). It is clear from our results that EVOO and OVOO didn't have significant effects on creatinine, urea and uric acid concentration comparing with control. On the other hand, there were significant increases in these parameters in group G4 (55.46, 20.26 and 55.41%, respectively) compared to control normal group. Our results agreed with **Necib *et al.*, (2013)** who reported that virgin olive oil treatment did not show any significant alteration in serum urea, creatinine and uric acid levels which were noticed respect to controls.

In normal pathological states EVOO have been described as anti-oxidants that remove free radicals for cell protection **(Serreli and Deiana, 2020).** In experimental studies, olive oil phenolic compounds showed strong antioxidant properties against lipids, DNA and LDL oxidation **(Covas *et al.*, 2006a)**. Hydroxytyrosol (2-(3,4 dihydroxyphenyl) Ethanol, DPE), one of the phenolic compounds present in EVOO has been suggested to be a potent antioxidant, thus contributing to the beneficial properties of olive oil **(Deiana *et al.*, 1999)**. DPE administration has been shown to reduce the consequences of passive smoking-induced oxidative stress **(Visioli *et al.*, 2000),** prevent LDL oxidation **(Wiseman *et al.*, 1996)** and platelet aggregation and inhibit leukocyte 5- lipoxygenases **(De la Puerta *et al.*, 1999)**. Virgin olive oil as an antioxidant agent, ameliorated oxidative injury in the tissues and functional deteriorated. Each clinical event is perceived by tissue macrophages and monocytes, which in turn secrete cytokines such asinterleukine1, interleukin 6 and TNFα **(Ziembia *et al.*, 2005),**

indicating the role of this cytokine in this toxicity, while virgin olive oil depressed the IL1, IL6 and TNFα levels response. Our results in group G4 were in agreement with **Stevens *et al.*, (2008)** who reported that, there are serious concerns on potential risks of this type of diet for renal function because of the trans-fatty acids.

Effect of olive oil categories on erythrocyte (SOD, Catalase, GSPx and GSH) and serum (MDA) levels in rats were recorded in Table (8).

Under normal conditions, there is a natural defense system provided by several enzymes such as (SOD), (CAT), (GSPx) and non-enzymatic antioxidants (GSH) which performs a vital role for detoxification of free radicals. The use of antioxidant rich food such as virgin olive oil or antioxidant food supplements became immensely popular since many diseases have been associated with oxidative stress **(Nakbi *et al.*, 2010).** Among these antioxidants from extra-virgin OO, phenolic compounds have received the most attention. Oleuropein derivatives, especially hydroxytyrosol, have been shown to have protective effects against markers associated with the atherogenic process **(Bondia-Pons *et al.*, 2007; Andreadou *et al.*, 2006 and Gonzalez-Santiago *et al.*, 2006)** and to have an antioxidant capacity higher than that of other known antioxidants such as vitamins E and C **(Paiva-Martins and Gordon, 2005)**.

Table (8): Effect of olive oil categories on erythrocyte (SOD, Catalase, GSPx and GSH) and serum (MDA) levels in rats

Treatments	SOD (U/ml)	Catalase (U/ml)	GSPx (U/ml)	GSH (mg/dl)	MDA (nmol/ml)
G1	$277.80^{b}\pm0.800$	$170.10^{b}\pm2.100$	$179.90^{b}\pm1.900$	$37.25^{b}\pm2.850$	$4.99^{b}\pm0.700$
G2	$290.60^{a}\pm1.650$	$179.9^{a}\pm1.850$	$184.04^{a}\pm2.450$	$40.70^{a}\pm4.700$	$4.05^{d}\pm0.550$
G3	$279.72^{b}\pm2.350$	$172.86^{b}\pm2.650$	$181.07^{b}\pm4.700$	$38.25^{b}\pm3.250$	$4.50^{c}\pm0.650$
G4	$198.68^{c}\pm3.600$	$154.30^{c}\pm4.300$	$149.81^{c}\pm3.150$	$29.70^{d}\pm4.700$	$5.80^{a}\pm0.800$
LSD	2.128	2.980	2.180	1.737	0.18853

- Means, within the same column, followed by the same letter are not significantly different at <0.05.
- Means are followed by the corresponding standard deviation
- G1: fed on basal diet, G2: fed on EVOO, G3: fed on OVOO and G4: fed on OO (Mixture of virgin olive oil and refined olive oil)

Table (8) showed the activity levels of (MDA) in serum, enzymatic antioxidants, (SOD), (CAT), (GSPx) and non-enzymatic antioxidants (GSH) in blood in normal and experimental rat groups. EVOO (G2) significantly decreased the levels of (MDA) in serum and significantly increased the activities of enzymatic antioxidants (SOD), (CAT), (GSPx) and non-enzymatic antioxidants (GSH) in blood (18.84, 4.61, 5.76, 2.30, 9.26%, respectively) in contrary with normal control group. Also, ordinary olive oil slightly elevated the activities of enzymatic antioxidants and non-enzymatic antioxidants without significant differences. These results agreed with **Oliveras-López *et al.*, (2008)** who indicate that, in metabolic tissues, protection by EVOO against oxidative stress occurs primarily through a direct antioxidant effect as well as through an indirect mechanism that involves greater expression and activity of certain enzymes with antioxidant activities. On the other hand, group G4 had significantly increase in the levels of (MDA) in serum and significantly decreases in the activities of enzymatic antioxidants (SOD), (CAT), (GSPx) and non-enzymatic antioxidants (GSH) in blood (16.23, 28.48, 9.29, 16.73, and 20.27%, respectively) with respect to control group. The studies of total antioxidant capacity (TAC) attempted to evaluate general levels of antioxidants in plasma **(Friedman *et al.*, 2003),** finding decreased TAC in animals exposed to oxidative stress. Tissues

with low levels of the reduced form of (GSH) are much more sensitive to external oxidative injuries **(Puiggro´s *et al.*, 2005),** as cells exposed to higher oxidation exhibit accumulation of oxidized glutathione (GSSG). (MDA) accumulation results from membrane peroxidation **(Kim *et al.*, 2000)**. Therefore, quantification of MDA accumulation provides insight into the integrity of lipid membranes. (CAT), (SOD), and (GTPX) are considered some of the more important antioxidant defenses of the organism against the production of free radicals **(Puiggro´s *et al.*, 2005)**. The low level of expression of antioxidant enzymes these enzymes have been considered the main reason for the susceptibility of cell to oxidative stress **(Lenzen *et al.*, 1996)**; therefore, it was predicted that increasing the expression of these enzymes would impart a beneficial effect of antioxidant protection. A stressful condition leads to the excessive production of free radicals which results in oxidative stress an imbalance in the oxidant per antioxidant system. Generation of free radicals is an integral feature of normal cellular functions in contrast to excessive generation and/or inadequate removal of free radical results in destructive and irreversible damage to the cell (**Lobo *et al.*, 2010)**.

Histopathological examination of liver rats in different experimental groups were showed in Figures (1 & 2). The microscopic examined sections of the liver of the experimental rats of groups G1, G2 and G3 (Fig.1) showed a normal histological appearance of hepatic lobule (slide1, 2, 3, 4, 5 and 6). Also, liver section of rat from group G4 (Fig.2) showed no histopathological changes hepatocytes (slide7). Whereas, other sections showed slight activation of Kupffer cells (slide8), congestions of central vein and small focal hepatocellular necrosis associated with inflammatory cells infiltration (slide9), hepatocellular vacuolization (slide10), focal hepatocellular necrosis associated with inflammatory cells infiltration (slide11) and portal infiltration with inflammatory cells (slide12).

Conclusion

Our study revealed that EVOO had the best biological results for all parameters. Moreover, the results also proved that there were no negative effects for consumption of OVOO and it was clear from the assessed biological parameters comparing with other categories, unlike OO (containing refined olive oil) which caused significant increases in TC, LDL, VLDL, TG and MAD levels and also increased ALT, AST, ALP, SOD, CAT, GSPx activity whereas, HDL and GSH levels were decreased. Also, OVOO did not show any histopathological alterations in examined liver sections.

From the biological results in our study, we can conclude that consumption of OVOO had no any health implications or harm effects on health. Maybe, it has low quality category compared to EVOO. This issue not related to food safety. So, we recommended not to remove OVOO from Codex Standard 33-1981.

Acknowledgment

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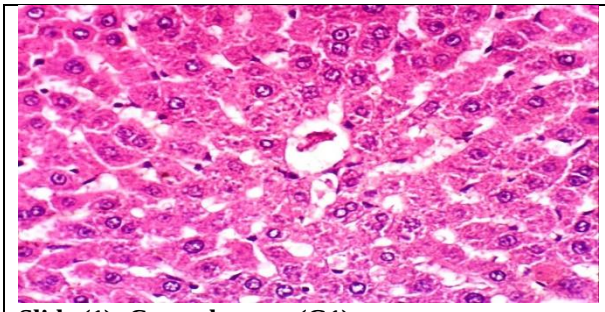
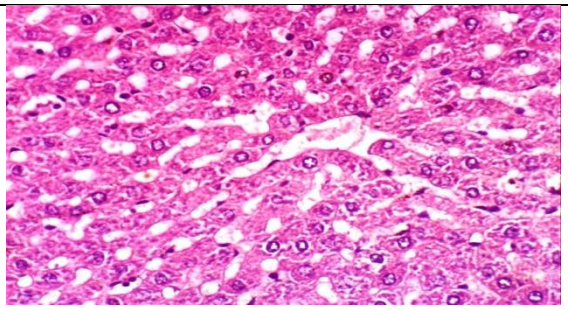


Slide (1): Control group (G1)



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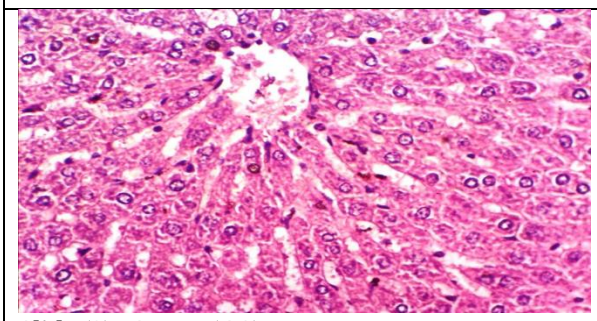

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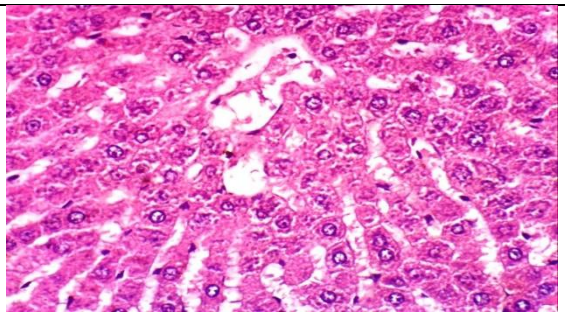

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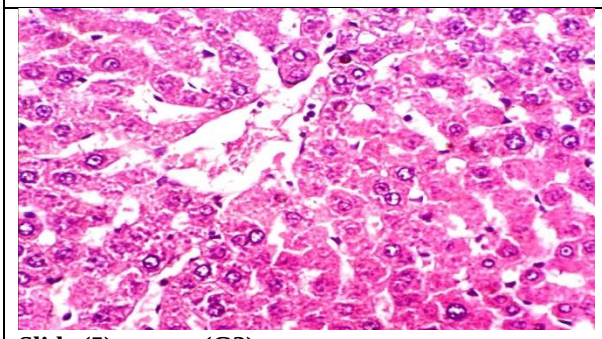
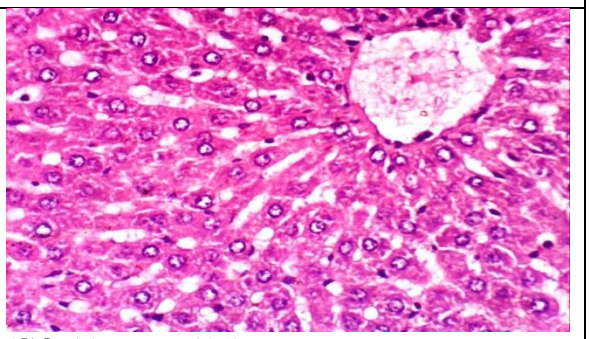
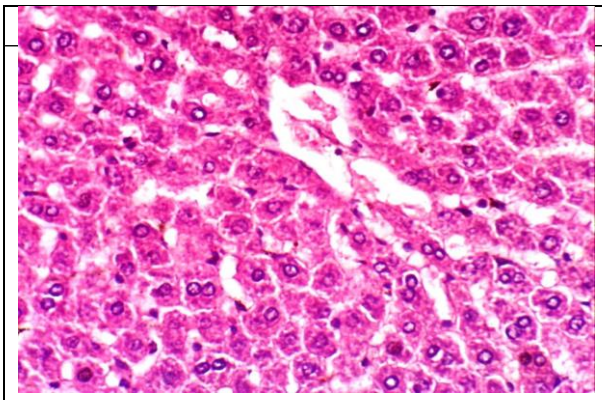


Slide (5): group (G3)

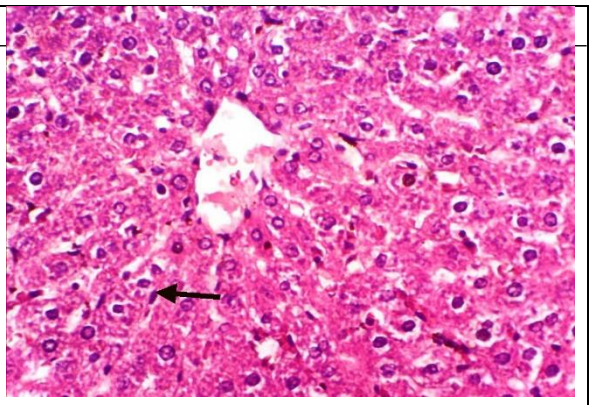


Slide (6): group (G3)

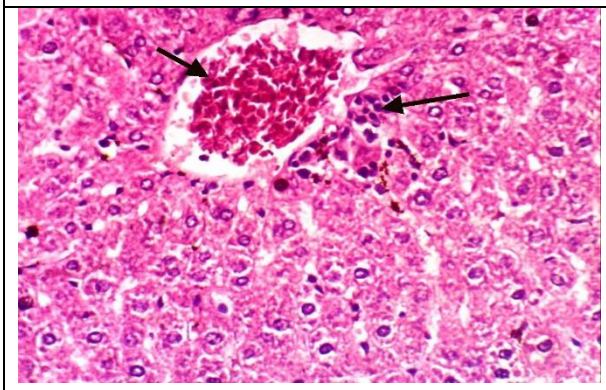
Figure (1): Liver sections of rats from group G1, G2 and G3showing no histopathological alterations (H & E X 400).



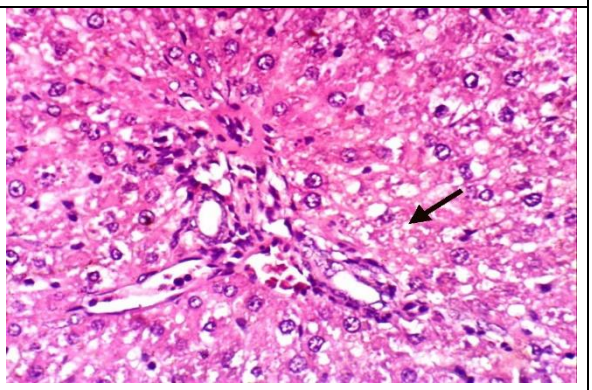
Slide (7): Liver section of rat from group G4 showing no histopathological alterations (H & E X 400)



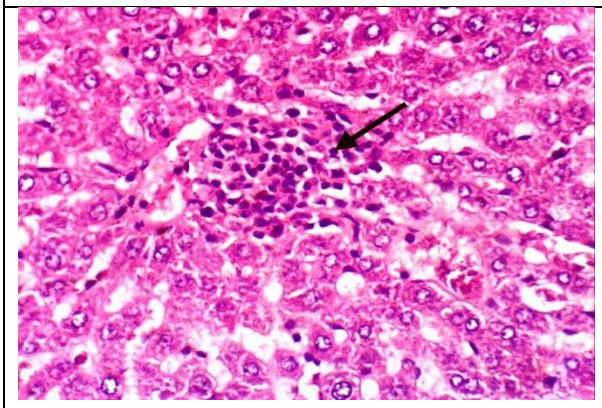
Slide (8): Liver of rat from group G4 showing slight activation of Kupffer cells (H & E X 400).



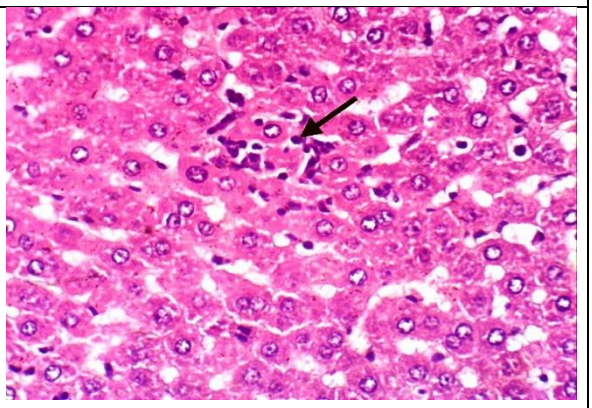
Slide (9): Liver of rat from G4 showing congestion of central vein and small focal hepatocellular necrosis associated with inflammatory cells infiltration (H & E X 400).



Slide (10): Liver of rat from G4 showing hepatocellular vacuolization (H & E X 400).



Slide (11): Liver of rat from G4 showing focal hepatocellular necrosis associated with inflammatory cells infiltration (H & E X 400)



Slide (12): Liver of rat from G4 showing sinusoidal leukocytosis (H & E X 400).

Figure (2): Histopathological changes in tissue sections livers rats of groups G4

REFERENCES

Abril, D., Mirabal-Gallardo, Y., González, A., Marican, A., Durán-Lara, E. F., Silva Santos, L. and Valdés, O. (2019). Comparison of the oxidative stability and antioxidant activity of extra-virgin olive oil and oils extracted from seeds of Colliguaya integerrima and Cynara cardunculus under normal conditions and after thermal treatment. Antioxidants, 8, 470.https:// doi: 10.3390/antiox8100470

Aebi, H., (1984). Catalase in vitro. Methods Enzymol. 105:121-136.

Aletor, V.A., 1993. Cyanide in garri 2: Assessment of some aspects of nutritional, biochemistry and heamotology of the rats fed garri containing varying residual cyanide levels. Int. J. Food Sci. Nutr., 44: 289-292.

Allain, C.C.; Poon, L.S.; Chan, C.S.; Richmond, W. and Fu, P.C. (1974): Enzymatic determination of total serum cholesterol. Clinical chemistry, (20): 470- 475.

Andreadou I., Iliodromitis E. K., Mikros E., Constantinou M., (2006). The olive constituent oleuropein exhibits anti-ischemic, antioxidative, and hypolipidemic effects in anesthetized rabbits. J Nutr. 2006; 136:2213–9.

Aning, K.G., A.G. Ologun, A. Onifade, J.A. Alokan and V.A. Aletor, 1998. Effect of replacing dried brewer's grains with sorghum rootlets on growth, nutrient utilization and some blood constitutes in the rat, Anim. Feed Sci. Tec., 71: 185-190.

AOAC (2019). Official Methods of Analysis of the Association of Official Analytical Chemists: Official Methods of Analysis of AOAC International. 21st Edition, AOAC, Washington DC.

Armin S, Taleban FA, Tahbaz F, Mehrabi Y, Kamali Z. (2010). Comparison of the effects of consuming olive and sunflower oils and lipids and fasting blood sugar levels after breakfast in postmenopausal women with type 2 diabetes. Iran J Nutr Sci Food Technol 2010; 4:75–83.

Arrigo T, Leonardi S, Cuppari C, Manti S, Lanzafame A, D'Angelo G, Gitto E, Marseglia L, Salpietro C. (2015). Role of the diet as a link between oxidative stress and liver diseases. World J Gastroenterol. 2015 Jan 14;21(2):384-95. doi: 10.3748/wjg. v21.i2.384. PMID: 25593454; PMCID: PMC4292270.

Assmann, G. (1979). A fully enzymatic colorimetric determination of HDL- cholesterol in the serum. Internist, 20, 559-565. https:// doi: 10.21608/EJCHEM.2020.37364.2792

Atefi M, Pishdad GR, Faghih S. (2018). Canola oil and olive oil impact on lipid profile and blood pressure in women with type 2 diabetes: a randomized, controlled trial. Progr Nutr [Internet]. 2018 Aug. 6 ;20(1-S):102-9.

Basuny, M.A., M.S. Arafat and M.D. Mostafa, (2008). Virgin olive oil quality: Relationship between bioactive components and organoleptic evaluation. Alex. J. Fd. Sci.& Tech., 3: 21-29.

Beltran, G., (2000). Influence of Ripening Process in Olea europaea L. Fruits on the Physicochemical Characteristics of the Oils. Ph.D. Thesis, Universidad de Jaen, Spain

Bergmeyer, H. U. and Harder, M., (1986). A colorimeteric method of determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Clin. Biochem., 24: 28 - 34.

Berrougui H., Cloutier M., Isabelle M., and Khalil A., (2006). "Phenolic extract from argan oil (Argania spinosa L.) inhibits human low-density lipoprotein (LDL) oxidation and enhances cholesterol efflux from human THP-1 macrophages," Atherosclerosis, vol. 184, no. 2, pp. 389–396, 2006.

Blanco-Molina A., Castro G., Mart´ın-Escalante D., et al., (1998). "Effects of different dietary cholesterol concentrations on lipoprotein plasma concentrations and on cholesterol efflux from Fu5AH cells," The American Journal of Clinical Nutrition, vol. 68, no. 5, pp. 1028–1033, 1998**.**

Bondia-Pons I., Schro¨der H., Covas M. I., Castellote A. I., (2007). Moderate consumption of olive oil by healthy European men reduces systolic blood pressure in non-mediterranean participants. J Nutr. 2007; 137:84–7.

Borello, E. and Domenici, V. (2019). Determination of Pigments in Virgin and Extra-Virgin Olive Oils: A Comparison between Two Near UV-Vis Spectroscopic Techniques. Foods 2019, 8(1): 1-13.

Boskou G., Salta F. N., Chrysostomou S., Mylona A., Chiou A., Andrikopoulos N. K. (2006). Antioxidant capacity and phenolic profile of table olives from the Greek market. Food Chem 94: 558– 64.

Browning J. D. and Horton J. D., (2004). Molecular mediators of hepatic steatosis and liver injury. J Clin Invest 2004, 114:147-152. 22.

Caraway, W. T. (1955). Determination of uric acid in serum by a carbonate method. American Journal of Clinical Pathology 25: 840-845.

Carluccio M. A., Massaro M., Scoditti E., and De Caterina R. (2007). "Vasculoprotective potential of olive oil components," Molecular Nutrition and Food Research, vol. 51, no. 10, pp. 1225–1234, 2007.

CCFO 25th, (2017), Report of the 25th session of the codex committee on fats and oils. Kuala, Malaysia. 27 February – 03 March.

CCFO 26th, (2019), Report of the 26th session of the codex committee on fats and oils. Kuala Lumpur, Malaysia. 25 February – 01 March.

Chapman, D.; Castilla, R. and Cambell, L. (1959). Evaluation of protein in food: A method for the determination of feed efficiency ratio. Can. J. of Biochemical Physicol. ,37; 679 -686.

Codex Standard (1981). STANDARD FOR OLIVE OILS AND OLIVE POMACE OILS. CXS 33-1981

Codex Alimentarius 8 (2003) 25-39**.**

Codex Standard (1999) for Named Vegetable Oils (CXS 210-1999) Amended 2019.

Cossiganani, L., Simonetti, M.S. and Damiani, P. (2005). Biocatalyzed acidolysis of olive oil triacylglycerols with 9c,11t and 10t,12c isomers of conjugated linoleic acid Eur. Food Res. Technol., 220, 267-271 (2005).

Covas M. I., De La Torre K., Farre-Albaladejo M., et al., (2006a). Postprandial LDL phenolic content and LDL oxidation are modulated by olive oil phenolic compounds in humans. Free Radical Biology & Medicine, vol. 40, no. 4, pp. 608–616, 2006.

Covas M. I., Nyyssonen K., Poulsen H. E., et al., (2006b). "The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial," Annals of Internal Medicine, vol. 145, no. 5, pp. 333– 341, 2006.

De la Puerta, R., Ruiz Gutierrez V. and Hoult J.R.S., (1999). Inhibition of leukocyte 5-lipoxygenase by phenolics from virgin olive oil. Biochemical Pharmacol., 57: 445-449. DOI: 10.1016/S0006-2952(98)00320-7

Deiana, M., Aruoma O.I., Bianchi M.D.L.P., Spencer J.P. and Kaur H., et al., (1999). Inhibition of peroxynitrite dependent DNA base modification and tyrosine nitration by the extra virgin olive oil-derived antioxidant hydroxytyrosol. Free Radical Biol. Med., 26: 762-769. DOI: 10.1016/S0891-5849(98)00231-7

Dhibi M., Brahmi F., Mnari A., Houas Z., Chargui I., Bchir L., Gazzah N., Alsaif M., Hammami M. (2011). The intake of high fat diet with different trans fatty acid levels differentially induces oxidative stress and non-alcoholic fatty liver disease (NAFLD) in rats. Nutr. Metab. 2011; 8:65.

Echeverria F., Ortiz M., Valenzuela R., Videla L.A. (2017). Hydroxytyrosol and Cytoprotection: A Projection for Clinical Interventions. Int. J. Mol. Sci. 2017; 18:930. doi

EEC. (1991). Characteristics of Olive and Olive Pomace Oils and their Analytical Methods. Regulation EC/2568/91 and Latter Modification. Official Journal of the European Communities.

Eid, M. M. and M. E. I. Elsorady, (2012). Sterols compositions of some olive oil varieties cultivated under Egyptian conditions. Egypt. J. Agric. Res., 90 (1) 263-276.

Ellman, G.L., (1959). Tissue sulfhydrl groups. Archieves of Biochem. and Biophys., 82: 70–77.

El-Sharouny, S. H. R., A. A. E.; Shawky,S. A. T. M. and Bayoumy, A. H. (2016). Acrylamide induced liver toxicity and the possible protective role of vitamine E in adult male albino rat: histological, biochemical and histochemical study. The Egyptian Journal of Medical Sciences, 37, 727-745.

Esfarjani F, Mohammadi F, Khalafi M, Roustaee R. (2013). Olive Oil in the Diet of Obese Children. Jfst 2013; 13:11–7.

Fawcett, J. K. and Scott, J. E., (1960). Enzymatic colorimetric method for determination urea in serum, plasma and urine. J. Clin. Path., 13,156-162.

Firestone, D., (2005). Olive oil: In Bailey's Industrial Oil and Fat Products, Sixth Edition, Six Volume Se**t.**

Foley, R. N.; Wang, C. and Collins, A. J. (2005). Cardiovascular risk factor profiles and kidney function stage in the US general population: the NHANES III study. Mayo Clinic Proceedings .80(10):1270-1277.

Food, Board Nutrition Institute of medicine of the national academies. National Academies Press; 2005. p. 423.

Fossati, P. and Prencipe, L., (1982). The determination of triglycerdes using enzymatic methods. Clin. Chem., 28: 2077 – 2081.

Friedman J., Peleg E., Kagan T., Shnizer S. and Rosenthal T. (2003). Oxidative stress in hypertensive, diabetic and diabetic hypertensive rats. Am J Hypertens. 2003; 16:1049–52.

Gonzalez-Santiago M., Martin-Bautista E., Carrero J. J., Fonolla J., Baro L., Bartolome M. V., Gil-Loyzaga P., Lopez-Huertas E. (2006). One month administration of hydroxytyrosol, a phenolic antioxidant recent in olive oil, to hyperlipemic rabbits improves blood lipid profile, antioxidant status and reduces atherosclerosis development. Atherosclerosis. 2006;188: 35–42. 4.

Goupy, P.,Hugues, M.,Biovin, P. & Amiot, M. J. (1999). Antioxidant composition and activity of barley (Hordeumvulgare) and malt extracts and of isolated phenolic compounds. J. Sci. Food Agric. 79:1625-1634.

Gutfinger, T. (1981). Polyphenols in Olive Oils. Journal of the American Oil Chemists Society, 58, 966-968. https://doi.org/10.1007/BF02659771

Halliwell B., (1997). Antioxidants and human disease: a general introduction. Nutr Rev 1997, 55:44-49.

Han H, Zhong R, Zhou Y, et al. (2022). Hydroxytyrosol Benefits Boar Semen Quality via Improving Gut Microbiota and Blood Metabolome. Front Nutr. 2022; 8:815922. Published 2022 Jan 17. doi:10.3389/fnut.2021.815922.

Hashempour, A., R.F. Ghazvini, D. Bakhshi and S.A. Sanam, (2010). Fatty acids composition and pigments changing of virgin olive oil (Olea europea L.) in five cultivars grown in Iran. AJCS 4(4): 258-263 ISSN:1835-2707.

Hassen I., Casabianca H., Hosni K. (2015). Biological activities of the natural antioxidant oleuropein: Exceeding the expectation – A mini-review. J. Funct. Food. 2015; 18:926–940.

Henry, R. J., (1974). Clinical Chemistry, Principles and Technichs, 2 nd Edition, Harper and Raw, p. 525.

Illesca P. G., Lavandera J. V., Gerstner C. D., González M. A., Bernal C. A. (2015). Trans fatty acids modify nutritional parameters and triacylglycerol metabolism in rats: Differential effects at recommended and high-fat level. Nutr Hosp. 2015 Aug 1;32(2):738-48. doi: 10.3305/nh.2015.32.2.9190. PMID: 26268106.

IOC (2018). International olive council: trade standard applying to olive oils and olive-pomace oils. (COT/T.15/NC3/Rev. 12.)

IOC (2019). International olive council: trade standard applying to olive oils and olive-pomace oils. COT/T.15/NC No 3/Rev. 14.

ISO (2016). International Organization for Standardization. (2016). Occupational health and safety management system-Requirement with guidance for use (ISO/DIS Standard No. 45001). Retrieved from http://www.iso.org/catalogue_detail?csnumber=63787

Kaplan, L.A. (1984). Clinical Chemistry. The C.V. Mosby Co. St Louis. Toronto. Princeton, 1032-1036.

Karković Marković, A., Torić, J., Barbarić, M., & Jakobušić Brala, C. (2019). Hydroxytyrosol, Tyrosol and Derivatives and Their Potential Effects on Human Health. Molecules (Basel,Switzerland), 24(10),2001.

Khlil, E., Mansouri, F., Ben moumen, A., Serghini-Caid, H., Berrabah, M., & Tahri, E. (2017). Physicochemical characteristics of monovarietal olive oil produced at Beni Tajjit, South-West of the region of eastern Morocco. Journal of Materials and Environmental Science, 8, 4264– 4272. https://doi.org/10.26872/jmes.2017.8.12.449

Kim J., Chehade J., Pinnas J. L. and Arshag D. M. (2000). Effect of select antioxidants on MDA modification of proteins. Nutrition. 2000; 16:1079–81.

Lee, R. and Nieman, D. (1996): Nutrition Assessment. 2nd Ed. Mosby Missouri, USA.

Lenzen S., Drinkgern J. and Tiedge M. (1996). Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. Free Radic Biol Med. 1996; 20:463–6.

Lobo, V., Patil, A., Phatak, A. and Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews*, 4, 118.https://doi: 10.4103/0973-7847.70902.

Longhi R, Almeida RF, Machado L, Duarte MMMF, Souza DG, Machado P, de Assis AM, Quincozes-Santos A, Souza DO. (2017). Effect of a trans fatty acid-enriched diet on biochemical and inflammatory parameters in Wistar rats. Eur J Nutr. 2017 Apr;56(3):1003-1016. doi: 10.1007/s00394-015-1148-y. Epub 2016 Jan 11. PMID: 26754301.

Macfarlane I., Bomford A. and Sherwood R. A. (2000). Liver diseases and Laboratory Medicine.ACB Ventures Publications London.

Mailer R., (2006). Chemistry and quality of olive oil. PRIMEF ACT 227, pp: 1-4.

Malekmohammad K., Sewell R. D. and Rafieian-Kopaei M. (2019). Antioxidants and atherosclerosis: Mechanistic aspects. *Biomolecules,* 9, 301.

Maritim, A.C., Sanders, R.A. and Watkins, J.B. (2003). Diabetes, oxidative stress and antioxidants: a review, Journal of Biochemical and Molecular Toxicology, 17: 24–38, https://doi.org/10.1002/jbt.10058. Min-Chieh, H., Yi-Jie, P., Shih-Jen, C., Shih-Hw

Martensson J., Jain A. and Meister A. (1990). Glutathione is required for intestinal function. *Proceedings of the National Academy of Sciences,* 87, 1715-1719.https://https://doi: 10.1073/pnas.87.5.1715

Meltzer, H. M.; Folmer, M.; Wang, S.; Lie, Q.; Maage, A. and Mundal, H. H., (1997). Supplementary selenium influences the response to fatty acid induced oxidative stress in humans. Biological Trace Element Research, 60: 51-67.

Minguez-Mosqueera, M.I., Rejano, L., Gandul, B., Sanchez, A. H. and Garrido, J. (1991). Color-Pigment correlation in virgin olive oil.J. Am. Oil Chem. Sco., 86: 322-337.

Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC. (2006). Trans fatty acids and cardiovascular disease. N Engl J Med. 2006 Apr 13;354(15):1601-13. doi: 10.1056/NEJMra054035. PMID: 16611951.

Mozaffarian, D., Aro, A. & Willett, W. (2009). Health effects of *trans*-fatty acids: experimental and observational evidence. *Eur J Clin Nutr* **63,** S5–S21 (2009).

Myers, G. L.; Miller, W. G.; Coresh, J.; Fleming, J.; Greenberg, N.; Greene, T.; *et al.*, (2006). Recommendations for Improving Serum Creatinine Measurement: A Report from the Laboratory Working Group of the National Kidney Disease Education Program. *Clinical Chemistry*; 52:5-18.

Nakbi, A., Tayeb, W., Dabbou, S., Issaoui, M., Grissa, A. K., Attia, N. and Hammami, M. (2010). Dietary olive oil effect on antioxidant status and fatty acid profile in the erythrocyte of 2, 4-D-exposed rats. *Lipids in health and disease*, 9, 1-10.

Necib, Y., Bahi, A. Zerizer, S. Abdennour, C. and Boulakoud, M. S. (2013). Effect of Virgin Olive Oil (Olea europea. L) on Kidney Function Impairment and Oxidative Stress Induced by Mercuric Chloride in Rats. American Journal of Biochemistry and Biotechnology 9 (4): 415-422, 2013

Nishikimi, M., Rao, N. A. and Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and biophysical research communications, 46, 849-854.https:// doi.org/ 10.1016/ S0006-291X (72)80218-3

Nogoy KMC, Kim HJ, Lee Y, Zhang Y, Yu J, Lee DH, Li XZ, Smith SB, Seong HA, Choi SH. (2020) High dietary oleic acid in olive oil-supplemented diet enhanced omega-3 fatty acid in blood plasma of rats. Food Sci Nutr. 2020 May 19;8(7):3617-3625. doi: 10.1002/fsn3.1644. PMID: 32724624; PMCID: PMC7382191.

Oliveras-López M.J., G. Berna, E.M. Carneiro, H. López-García de la Serrana, F. Martin, M.C. López. (2008). An extra-virgin olive oil rich in polyphenolic compounds has antioxidant effects in OF1 mice J Nutr, 138 (2008), pp. 10.

Ozer, O. E.; Ucar, G.; Helvacioglu, F.; Akaydin-Aldemir, D. and Turkoglu, S. (2014). Effect of acrylamide treatment on arginase activities and nitric oxide levels in rat liver and kidney. Acta Medica Mediterranea, 30, 375-382.

Paiva-Martins F. and Gordon M. H. (2005). Interactions of ferric ions with olive oil phenolic compounds. J Agric Food Chem. 2005; 53:2704–9. 5.

Pardun, V. H. (1988). Desodorierung von Speiseölen im laboratoriumsmaßstab. Fat. SCi. Technol, 90, 5-14

Parkinson L., Cicerale S. (2016). The Health Benefiting Mechanisms of Virgin Olive Oil Phenolic Compounds. Molecules. 2016; 21:1734.

Perona J. S., Canizares J., Montero E., ănchez-Dom ´ ´ınguez J. M. S, Catala A., and Ruiz-Guti´errez V. (2004). "Virgin olive oil reduces blood ´ pressure in hypertensive elderly subjects," Clinical Nutrition, vol. 23, no. 5, pp. 1113–1121, 2004.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D.and Bitto, A. (2017). Oxidative stress: harms and benefits for human health. *Oxidative medicine and cellular longevity*, 2017.https://doi: 10.1155/2017/8416763

Puiggro´s F., Llo´ piz N., Arde´vol A., Blade´ C., Arola L., Salvado´ M.J., (2005). Grape seed procyanidins prevent oxidative injury by modulating the expression of antioxidant enzyme systems. J Agric Food Chem. 2005;53: 6080–6.

Raja, M., Al-Fatah, A., Ali, M., Afzal, M., Hassan, R., Menon, M. and Dhami, M. I. (1992). Modification of liver and serum enzymes by paraquat treatment in rabbits. *Drug metabolism and drug interactions*, 10, 279-292.

Reboredo-Rodríguez, P., González-Barreiro, C., Cancho-Grande, B., Fregapane, G., Salvador, M. D. and Simal-Gándara, J. (2015). Characterisation of Extra Virgin Olive Oils from Galician Autochthonous Varieties and Their Co-Crushings with Arbequina and Picual cv. Food Chem., 176: 493–503.

Robles-Almazan M, Pulido-Moran M, Moreno-Fernandez J, Ramirez-Tortosa C, Rodriguez-Garcia C, Quiles JL, Ramirez-Tortosa M. (2018). Hydroxytyrosol: Bioavailability, toxicity, and clinical applications. Food Res Int. 2018 Mar;105:654-667. doi: 10.1016/j.foodres.2017.11.053. Epub 2017 Nov 21. PMID: 29433260.

Rodriguez-Morato J., Xicota L., Fito M., Farre M., Dierssen M., de la Torre R. (2015). Potential Role of Olive Oil Phenolic Compounds in the Prevention of Neurodegenerative Diseases. Molecules. 2015; 20:4655–4680.

Rotruck. J.J.; Pope, A. L.; Ganther, H.E. and Swanson, A.B., (1973). Selenium: biochemical role as a component of glutathione peroxidase. Science, 179: 588–590.

Schwingshackl L., Lampousi A.M., Portillo M.P., Romaguera D., Hoffmann G., Boeing H. (2017). Olive oil in the prevention and management of type 2 diabetes mellitus: a systematic review and meta-analysis of cohort studies and intervention trials**.** Nutr Diabetes, 7 (2017), p. e2.

Serreli, G. and Deiana, M. (2020). Extra virgin olive oil polyphenols: modulation of cellular pathways related to oxidant species and inflammation in aging. Cells, 9, 478.https://doi: 10.3390/cells9020478

Servili, M., Sordini, B., Esposto, S., Urbani, S., Veneziani, G., Di Maio, I., Selvaggini, R. and Taticchi, A. (2014). Biological activities of phenolic compounds of extra virgin olive oil. Antioxidants, 3, 1-23.https://doi: 10.3390/antiox3010001.

Shidfar F., Bahrololumi S., Doaei S., Mohammadzadeh A., Gholamalizadeh M., and Mohammadimanesh A. (2018). The Effects of Extra Virgin Olive Oil on Alanine Aminotransferase, Aspartate Aminotransferase, and Ultrasonographic Indices of Hepatic Steatosis in Nonalcoholic Fatty Liver Disease Patients Undergoing Low Calorie Diet. Canadian Journal of Gastroenterology and Hepatology 2018, P1- 7.

Sirtori C. R., Gatti E., Tremoli E., et al., (1992). Olive oil, corn oil, and n-3 fatty acids differently affect lipids, lipoproteins, platelets, and Superoxide formation in type II hypercholesterolemia. American Journal of Clinical Nutrition, vol. 56, no. 1, pp. 113–122, 1992.

Snedecor, G.W. and Cochran, W.G., (1980). Statistical methods. 7th Ed., p. 420. Iowa Stat. Univ. Press, Ames, Iowa, USA.

Sofi F, Macchi C, Abbate R, Gensini GF, Casini A. (2013). Mediterranean diet and health. Biofactors. 2013, 39 (4): 335-342.

Stevens V.A., Saad S., Poronnik P., Fenton-Lee C. A., Polhill T. S., Pollock C. A., (2008). The role of SGK-1 in angiotensin II-mediated sodium reabsorption in human proximal tubular cells. Nephrol Dial Transplant 23: 1834 – 1843, 2008.

Stevens, L. A.; Coresh, J.; Greene, T. and Andrew, S. L., (2006). Assessing kidney function - measured and estimated glomerular filtration rate. N.England Journal of Medicine, 354:2473-2483.

Terés S, Barceló-Coblijn G, Benet M, Alvarez R, Bressani R, Halver JE, et al. (2008). Oleic acid content is responsible for the reduction in blood pressure induced by olive oil. Proc Natl Acad Sci U S A 2008; 105:13811–6. doi:10.1073/ pnas.0807500105.

Tome-Carneiro J., Crespo M.C., Iglesias-Gutierrez E., Martin R., Gil-Zamorano J., Tomas-Zapico C., Burgos-Ramos E., Correa C., Gomez-Coronado D., Lasuncion M.A., et al. (2016). Hydroxytyrosol supplementation modulates the expression of miRNAs in rodents and in humans. *J. Nutr. Biochem.* 2016; 34:146–155.

Tripoli, E., Giammanco, M., Tabacchi, G., Di Majo, D., Giammanco, S. and La Guardia, M. (2005). The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. *Nutrition research reviews*, 18, 98-112.http://doi: 10.1079/NRR200495.

Varley, H.; Gewenlock, A. and Bell, M., (1980). Practical Clinical Biochemistry. vol. 1. 5thed., pp. 741, 897. London: William Heinemen Medical. Books, Ltd

Videla L. A., Rodrigo R., Araya J., Ponichik J., (2006). Insulin resistance and oxidative stress interdependency in non-alcoholic fatty liver disease. Trends Mol Med 2006, 12:555-558.

Videla LA, Rodrigo R, Araya J, Poniachik J., (2004). Oxidative stress and depletion of hepatic longchain polyunsaturated fatty acids may contribute to nonalcoholic fatty liver disease. Free Radic Biol Med 2004, 37:1499-1507. 21.

Visioli F., Poli A., Galli C. (2002). Antioxidant and other biological activities of phenols from olives and olive oil. *Med. Res. Rev.* 2002; 22:65–75.

Visioli, F., Galli C., Plasmati E., Viappiani S. and Hernandez A. et al., (2000). Olive phenol hydroxytyrosol prevents passive smoking–induced oxidative stress. Circulation, 102: 2169-2171. DOI: 10.1161/01.CIR.102.18.2169

Wallach, J. (1992). Interpretation of diagnostic tests. A synopsis of aboratory medicine, 5 th Ed. A little brown handbook, Boston.vv.

Wang M, Zhang S, Zhong R, Wan F, Chen L, Liu L, et al. (2021). Olive fruit extracts supplement improve antioxidant capacity via altering colonic microbiota composition in mice. *Front Nutr.* (2021) 8:645099.

Wani T.A., Masoodi F.A., Gani A., Baba W.N., Rahmanian N., Akhter R., Wani I.A., Ahmad M. (2018). Olive oil and its principal bioactive compound: Hydroxytyrosol—A review of the recent literature. *Trends Food Sci. Technol.* 2018; 77:77–90.

Wiseman S.A., Mathot J.N., de Fouw N.J., Tijburg L.B. (1996). Dietary non-tocopherol antioxidants present in extra virgin olive oil increase the resistance of low-density lipoproteins to oxidation in rabbits. Atherosclerosis. 1996 Feb;120(1-2):15-23. doi: 10.1016/0021-9150(95)05656-4. PMID: 8645356.

Yoon, B. I.; Choi, Y. K.; Kim, D. Y.; Hyun, B. H.; Joo, K. H.; Rim, H. J. and Lee, J. H. (2001). Infectivity and pathological changes in murine clonorchiasis: Comparison in immunocompetent and immunodeficient mice. J. Vet. Med. Sci., 63(4): 421-425.

Ziembia, S.E., McCabe J.R. and Rosenspire A.J., (2005). Inorganic mercury dissociates preassembled Fas/CD95 receptor oligomers in T lymphocytes. Toxicol. Applied Pharmacol., 206: 334-342. DOI: 10.1016/j.taap.2004.11.014

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